OIPE JC95 JUL 28 2004 PATENT & TRADEMARK OFFICE

S/N 10/798,199 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	ANAND et al.	Examiner:	unknown
Serial No.:	10/798,199	Group Art Unit:	unknown
Filed:	10 March 2004	Docket No.:	11378.58US01
Title:	STEREOSELECTIVE CHEMOENZYMATIC PROCESS FOR THE PREPARATION OF OPTICALLY ENRICHED PHENYLGLYCIDATES AS PRECURSORS OF TAXOL SIDE CHAIN		

CERTIFICATE UNDER 37 CFR 1.10
'Express Mail' mailing label number: EV372674209US
Date of Deposit: 28 July 2004
I hereby certify that this correspondence is being deposited with the United States Postal Service 'Express Mail Post Office To Addressee' service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner for Patents, Alexandria, VA 22313-1450

By: _____
Name: David Ortiz

SUBMISSION OF PRIORITY DOCUMENT(S)

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Applicants enclose herewith one certified copy of a Indian application, Serial No. 275/Del/2003, filed 12 March 2003, the right of priority of which is claimed under 35 U.S.C. § 119.

Respectfully submitted,

MERCHANT & GOULD P.C.
P.O. Box 2903
Minneapolis, Minnesota 55402-0903
(612) 332-5300

Dated: 28 July 2004

By _____
Douglas P. Mueller
Reg. No. 30,300

DPM:hjm

THIS PAGE BLANK (USPTO)

BEST AVAILABLE COPY

1817

11318.58US01

सत्यमेव जयते

INTELLECTUAL PROPERTY INDIA

GOVERNMENT OF INDIA
MINISTRY OF COMMERCE & INDUSTRY,
PATENT OFFICE, DELHI BRANCH,
W - 5, WEST PATEL NAGAR,
NEW DELHI - 110 008.

I, the undersigned being an officer duly authorized in accordance with the provision of the Patent Act, 1970 hereby certify that annexed hereto is the true copy of the ***Application, Complete Specification*** *and* ***Drawing Sheets*** *filed in connection with Application for Patent No.275/Del/03 dated* ***12th March 2003.***

Witness my hand this 22nd day of April 2004.

(S.K. PANGASA)
Assistant Controller of Patents & Designs

CERTIFIED COPY OF PRIORITY DOCUMENT

THIS PAGE BLANK (USPTO)

Chemistry
45/00

0275-DEL-2003

Indian Patent Office
New Delhi
Received Rs. 5000/- in cash.
cheque/M.O./I.P.O./D.D.
12 MAR 2003
Vide Entry No. 1093 in the Register of Valuables
A. O ..... Cashier

FORM 1 12 MAR 2003
THE PATENT ACT, 1970
(39of 1970)
APPLICATION FOR GRANT OF PATENT
(See Sections 5(2), 7, 54 and 135 and rule 33A)

We COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH, Rafi marg, New Delhi-110 001, India, an Indian registered body incorporated under the Registration of Societies Act (Act XXI of 1860) and PG Department of Chemistry, University of Jammu, Jammu having its campus at University of Jammu, Jammu-180 001

2. hereby declare:
(c) that we are in possession of an invention titled : A stereoselective chemoenzymatic process for the preparation of optically enriched phenylglycidates as precursors of Taxol side chain

(d) that the ~~Provisional~~/Complete specification relating to this invention is field with this application;(c) that there is no lawful ground of objection to the grant of patent to us;

2. further declare the inventor (s) for the said invention is/are;

1. NAVEEN ANAND
2. MUNISH KAPOOR
3. SUBHASH CHANDRA TANEJA
4. SURRINDER KOUL
5. RATAN LAL SHARMA
6. GHULAM NABI QAZI

, All are from RRL, Jammu, All are Indian citizens.

ORIGINAL

4. We, claim the priority from the application (s) filed in convention countries, particulars of which are as follows:
NOT APPLICABLE

5. We state that the said invention is an important in or modification of the invention, the particulars of which are as follows and of which we are the applicant:
(e) Patent application no:
(f) Patent application date:

6. We state that the application is divided out of our application the particulars of which are given below and pray that this application deemed to have been filed on .....under section 16 of the Act.
(e) Patent application no:
(f) Date of filling provisional and/or complete specification .....and .....

7. That we are the assignee of the true and first inventor (s)

8. That our address for services in India is an follows:
Head, IPM Division, CSIR,
INSDOC Building, 14 Satsang Vihar Marg,
New Delhi-110 067.

Phone :2696 2560, 2696 8819;

Fax :2696 8819

9. Following declaration was given by the inventor (s)
I/We the true and first inventor(s) for this invention declare that the applicants herein is / are my/ our assigne:

Date this 11th day of March, 20 03

Name (in full with expanded initials)	(Signature of the true and first inventor(s)
NAVEEN ANAND	Naveen Anand
MUNISH KAPOOR	Munish Kapoor
SUBHASH CHANDRA TANEJA	Subhash Chandra Taneja
SURRINDER KOUL	Surrinder Koul
RATAN LAL SHARMA	Ratan Lal Sharma
GHULAM NABI QAZI	Ghulam Nabi Qazi

10. That to the best our knowledge, information and belief the fact and matters stated herein are correct and that there is no lawful ground of patent to us on this application.

11. Followings are the attachment with application:

(m) ~~Provisional~~ / Complete specification (3 copies).

(n) Drawings (3 copies).

(o) Priority document(s).

(p) Statement and Undertaking on FORM-3.

(q) Power of authority.

(r) Fee Rs. 5000/- in Cheque no. 867716 date: 17/2/03 Rs/- 5,000
On State Bank of India, New Delhi Main Branch, Parliament Street, New Delhi – 110 001.

We request that a patent may be granted to us for the said invention.

Date this 11th day of March, 20 03

(Head of Department
Head of Department
PG Department of Chemistry
University of Jammu
JAMMU-180004

(SCIENTIST
Intellectual Property Management Divison,
Council of Scientific and Industrial

To,
The Controller of Patents,
The Patent Office, New Delhi

डा. श्रीमति इन्द्रा त्रिवेदी
Dr. (Smt.) Indra Trivedy
वैज्ञानिक/Scientist
आई.पी.एम.डी. (सी.एस.आई.आर.)
I.P.M. Division (C.S.I.R.)
४, सत्संग विहार मार्ग,
4, Satsang Vihar Marg,
नई दिल्ली-११००६७
New Delhi-110067

E 42/03

Form 2
COMPLETE SPECIFICATIONS

0275-DEL-03

(See section 10)

12 MAR 2003

A Stereoselective chemoenzymatic process for the preparation of optically enriched phenylglycidates as precursors of Taxol side chain

COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH
Rafi Marg, New Delhi-110001, India
An Indian body incorporated under the registration of societies act (XXI of 1860)

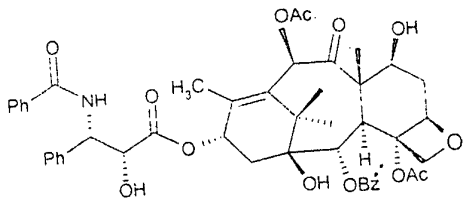
ORIGINAL

The following specifications particularly describe and ascertain the nature of this invention and the manner in which it is to be performed

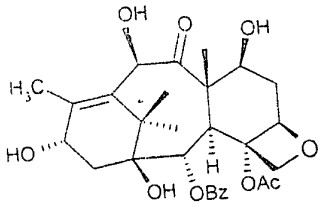
The present invention relates to a novel and efficient chemoenzymatic process of preparation of optically active trans alkyl phenylglycidates. The invention particularly discloses a novel process for the chemoenzymatic synthesis of two enantiomers of trans alkyl phenylglycidate i.e. alkyl(2*S*,3*R*)-phenylglycidate and alkyl(2*R*,3*S*)-phenylglycidate of formulae 7 and 8 respectively, process of their synthesis comprises cohalogenation reaction of alkyl cinnamate of formula 1 where R' represents C-1 to C-5 alkyl group to form trans 2-halo-3-hydroxy-3-phenylpropanoates of formula 2 where x represents Br or I, then converting the halohydrins of formula 2 to corresponding alkyl acylates of formula 3 where x and R' represents as above, subsequently incubating the acyl derivatives of alkyl 2-halo-3-hydroxy-3-phenylpropanoates of formula 3 with crude dry powder of lipase from *Aspergillus niger* in an aqueous buffer phase in presence of an organic solvent, thereafter separating the hydrolysed halohydrins i.e. alkyl(2R,3R) 2-halo-3-hydroxy-3-phenylpropanoates of formula 4 and unhydrolysed ester i.e. alkyl (2S,3S) 2-halo-3-acyloxy-3-phenylpropanoates of formula 5 from the mixture by conventional method, if required then again incubating the optically enriched acyl derivatives of formula 5 with crude dry powder of lipase from *Aspergillus niger* in an aqueous buffer phase in presence of an organic solvent to further improve the enantiopurity, followed by reaction of the optically enriched products of formula 5 with an acid to furnish optically enriched alkyl (2S,3S) 2-halo-3-hydroxy-3-phenylpropanoate of formula 6 and finally treating the compounds of formulae 4 and 6 with an alkali in an organic or aqueous phase to furnish optically enriched alkyl(2*S*,3*R*)-phenylglycidate and alkyl(2*R*,3*S*)-phenylglycidate of formulae 7 and 8 respectively .

Use of lipase from *Aspergillus niger* for the kinetic resolution of trans alkyl 2-halo-3-hydroxy-3-phenylpropanoates where x and R' represents as above is novel as also the step of acid followed by a base catalysed reaction for the cyclisation of resolved esters of trans alkyl 2-halo-3-hydroxy-3-phenylpropanoates of formula 4 and 6, not reported in the art of its synthesis. Optically enriched alkyl(2*S*,3*R*)-phenylglycidate and alkyl(2*R*,3*S*)-phenylglycidate of formulae 7 and 8 are the key intermediates used in the synthesis of N-benzoyl-(2*R*,3*S*)-3-phenylisoserine (Taxol side chain). Paclitaxel or Taxol

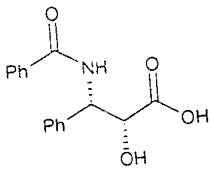
which was isolated from the bark of the Pacific Yew (*Taxus brevifolia*)(Wani *et al* *J Am Chem Soc.* 93, 2325-7 1971) and has been approved for the treatment of



Taxol

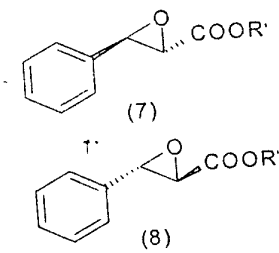


10-Deacetyl baccatin-III



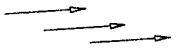
Isoserine

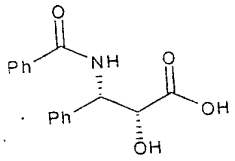
various types of cancers(Holmes *et al J. Natl. Cancer Inst.* 83, 1797-1805,1991). Despite being one of the most promising anti-cancer drugs, its very low occurrence (40-165 mg/kg) in nature is the main hindrance in its production. Fortunately it has been found that 10-deacetyl baccatin-III which is structurally closely related to Taxol and occurs in comparatively higher concentrations (approx. 1g/kg), can be easily isolated from fresh leaves of European Yew (*Taxus baccata*). It is also reported that Paclitaxel is 1000 times more potent compared to 10-deacetyl baccatin-III and its higher activity is due to a C-13 side chain comprising N-benzoyl-(2*R*,3*S*)-3-phenylisoserine moiety (Wani *et al J Am Chem Soc.* 93, 2325-7 1971).



(7)

(8)





Therefore synthesis of optically active (2*S*,3*R*) and (2*R*,3*S*)-phenylglycidates of formula 7 and 8 which are the key chiral precursors for synthesising N-benzoyl-(2*R*,3*S*)-3-phenylisoserine has become very vital for the development of a practical and efficient route to synthesise enantiomerically pure isoserine chain . There are number of reports on the preparation of these chiral intermediates by biochemical or chemoenzymatic methods besides asymmetric syntheses. Most of these reports are related to kinetic resolution of racemic 2-halo-3-hydroxy-3-phenylpropanoate through enzymatic hydrolysis (H.Honig *et al.*, *Tetrahedron* 46,

3841-3850, 1990; Peter G. M Wuts *et al, Tetrahedron Asymmetry* 11, 2117-2123, 2000), or by transesterification reaction (Ching-Shih Chen *et al., J. Org. Chem.* 58, 1287-1289, 1993; *Ching-Shih Chen et al,* U.S. Pat. No. 6,020,174, to The Board of Governors for Higher Education, Rhodes Island; Marco Villa *et al.,* U.S.Pat. No. 6,187,936 to Zambon Group S.p.A; Tanebe JP 06/078790) or via resolution of azetidinones (*C.J Sih et al., J. Org. Chem.* 58, 1068-1075, 1993; R.N. Patel *et al, J. American Oil Chemists Society,* 73, 1363-1375, 1996; R. A. Holton et al, WO 2001029245, EP 1222305 to Bristol-Myers Squibb; R.N. Patel *et al., Biotechnology and Applied Biochemistry,* 20, 23-33, 1994).

From the literature review it is quite clear that no prior art is available on kinetic resolution of racemic 2-halo-3-hydroxy-3-phenylpropanoates where x represents bromo and iodo groups and R' represents C-1 to C-5 alky group. Therefore the use of bromo and iodohydrins via kinetic resolution route for the preparation of desired (*2S,3R*) and (*2R,3S*)-phenylglycidates in essentially novel and has not been reported in the literature or known in the art of their synthesis of taxol side chain precursors.

The present invention therefore discloses the application of a lipase for the preparation of (*2S,3R*) and (*2R,3S*)-phenylglycidates via kinetic resolution of halohydrin intermediates x and R' are defined as above.

Thus the main objective of the present invention is to synthesise optically active alkyl (*2S,3R*) and (*2R,3S*)-phenylglycidates of formulae 7 and 8 using chemoenzymatic approach through resolution of its racemic precursor trans alkyl 2-halo-3-hydroxy-3-phenylpropanoates of formula 2 where x represents bromine or iodine and R' represenrts C-1 to C-5 alkyl group. Advantage of using bromo and iodohydrin intermediates are that they are easily preparable in almost quantitative yields and are obtained in crystalline form. Moreover these compounds can be easily converted to corresponding epoxides (glycidates) by acid and base catalysed transformations in almost quantitative yields. Additionally use of lipase from *Aspergillus niger* makes the process of resolution of trans alkyl 2-halo-3-hydroxy-3-phenylpropanoates facile.

The steps involved in resolution process are:

a. Preparation of halohydrins of formula 2 where x represents bromine or iodine and R' represents C-1 to C-5 alkyl group, from corresponding alkyl cinnamates of formula 1 by the action of a halogenating agent.

b. Chemical transformation of halohydrins of formula 2 to alkyl acylates of formula 3 using an acyl anhydride in presence of a base.

c. Incubating the trans alkyl 3-acyloxy-2-halo-3-phenylpropanoates of formula 3 with dry powder of the lipase from *Aspergillus niger* in an aqueous buffer phase in absence of an organic medium or in presence of an organic medium to facilitate the reaction. The bioresolution process is effected using the commercial enzyme lipase AMANO *AS* (*Aspergillus niger*) gifted by M/s AMANO, Japan and used directly without further purification or modification,

d. Separating the hydrolysed alkyl(2*R*,3R)-2-halo-3-hydroxy-3-phenylpropanoates of formula 4 and unhydrolysed alkyl(2*S*,3*S*)-3-acyloxy-2-halo-3-phenylpropanoates of formula 5 by conventional method of chromatography.

e. Reacting the optically enriched hydrolysed alkyl(2*R*,3*R*)-2-halo-3-hydroxy-3-phenylpropanoates of formula 4 with a base to furnish alkyl (2*S*,3*R*) phenylglycidates of formula 7.

f. Reaction of unhydrolysed alkyl(2*S*,3*S*)-3-acyloxy-2-halo-3-phenylpropanoates of formula 5 with an inorganic or organic acid to furnish corresponding alkyl(2*S*,3*S*)-2-halo-3-hydroxy-3-phenylpropanoates of formula 6. Further reacting the optically enriched hydrolysed alkyl(2*S*,3*S*)-2-halo-3-hydroxy-3-phenylpropanoates of formula 6 with a base to furnish alkyl(2*R*,3*S*) phenylglycidates of formula 8.

In a preferred embodiment of the process of preparation of halohydrins from alkyl cinnamates of formula 1 to halohydrins of formula 2 in step 'a' of the process, the halogenating agent is selected from N-halosuccinimide such as N-bromosuccinimide, N-iodosuccinimide or sodium bromate, periodic acid, 1,3-dibromo-5,5-dimethyl hydantoin, iodine, bromine and the like but more preferably sodium bromate for bromohydroxylation or periodic acid for iodohydroxylation in an aqueous phase or in a mixed organic aqueous phase

where organic solvent may be selected from water miscible solvents such as acetone, tetrahydrofuran, dioxane, acetonitrile and the like. The formation of halohydrins is carried out at a temperature in the range of 0-60°C more preferably at 30-40°C. In step 'b' of the transformation of compound of formula 2 to acylate of formula 3, the acylating agent is selected from acyl anhydrides such as acetic anhydride; propionic anhydride and butyric anhydride or corresponding acyl chlorides but most suitable is acetic anhydride in presence of bases like pyridine, N,N-dimethyl aminopyridine (DMAP) and more preferably DMAP.

In step 'c' of the process of kinetic resolution of the compound of formula 3 is effected by incubating the compound of formula 3 in presence of crude lipase enzyme (AS AMANO) from *Aspergillus niger* either in a buffered aqueous phase alone or a buffered aqueous phase in presence of an organic medium as a cosolvent which facilitates the hydrolytic reaction. The pH of the buffer is suitably adjusted at 5-7.5, more suitably at 6-7.5 and most suitably at 7. The temperature of the reaction is selected at 10-40°C, but more suitably at 20-35°C and most suitably at 30°C. The preferred aqueous phase is water, phosphate buffer (0.1M to 0.2M) or an acetate buffer and the most preferred one is phosphate buffer (0.1M). The preferred cosolvents that are added in the ratio of 10-90% are hexane, toluene, dichloromethane, acetone, acetonitrile, dimethylformamide, dimethyl sulphoxide, methanol, ethanol and the like. The more preferable are toluene, acetone and acetonitrile and most preferable is acetonitrile.

After the completion of the hydrolysis reaction in step 'd' of the process the separation of hydrolysed alcohol of formula 4 and unhydrolysed ester of formula 5 is effected by column chromatography on silica gel columns by conventional chromatographic methods.

In step 'e' of the process of base catalysed conversion of the compound of the formula 4 (hydrolysed ester) to alkyl(*2S,3R*)-phenylglycidates of the formula 7, is effected by an inorganic or organic base such as sodium hydroxide, sodium carbonate and the like and organic bases are selected from triethylamine, piperidine,1,4-diazabicyclo[2,2,2]octane (DABCO), 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) and the like, the more preferred base is DBU.

In step 'f' of the process of acid catalysed conversion of the compound alkyl (2*S*,3*S*)-3-acyloxy-2-halo-3-phenylpropanoates of the formula 5 (unhydrolysed ester) first to alkyl(2*S*,3*S*)-2-halo-3-hydroxy-3-phenylpropanoates of formula 6 is effected by mineral acid such as hydrochloric acid , sulfuric acid or an organic or lewis acid such as trifluoro acetic acid, boron trifluoride (BF_3) in an organic solvent such as diethyl ether, methanol, acetone and the like but the preferred acidic conditions are 1N, 2N and 5N HCl , most preferred is 2N HCl in methanol, finally transformation to alkyl(2*R*,3*S*)-phenylglycidates of the formula 8 is effected by a base as described in step 'e".

The invention is described herein with reference to the examples given below. These examples should not be construed as to restrict the scope of this invention.

Step 'a'

Example (i)

Synthesis of (±)-methyl 2-bromo-3-hydroxy-3-phenylpropanoate of formula 2 where x=Br and R'= CH_3

Potassium bromate (4g, 24 mmol), was dissolved in 40 ml water and adjusted to pH 1-2 with 2M H_2SO_4. To the resultant solution was added methyl cinnamate (3g, 20 mmol) in 40ml acetonitrile. 1M sodium bisulphite solution (5.2g in 50ml) was added to the above mixture over a period of two hours with stirring and at 40°C. The reaction mixture was further stirred for 36 hrs till the reaction was complete (TLC monitored). The resulting solution was extracted with ethyl acetate (3x100ml), and combined organic layer was washed with aqueous sodium sulphite followed by drying over anhydrous sodium sulphate. The contents concentrated in vacuo to give a crude material, which was purified by crystallization (benzene: hexane, 1:1) to furnish compound of formula 2 m.pt 63°C (yield 70%).

^{1}HNMR ($CDCl_3$) δ: 7.37(5H, s, Ar-H), 5.08(1H, d, *J*=8.25 Hz, CH-OH), 4.38(1H, d, *J*=8.24 Hz, CH-Br), 3.80(3H, s, $COOCH_3$).

Example (ii)

Synthesis of (±)-ethyl 2-bromo-3-hydroxy-3-phenylpropanoate of formula 2 where x=Br and R'= C_2H_5.

It was prepared from ethyl cinnamate (3.26g, 20 mmol) potassium bromate (4g, 24 mmol) following the procedure given in example step 'a' (i), m.pt 76-77°C, yield 3.7g, (70%).

^{1}HNMR($CDCl_3$) δ: 7.38(5H, s, Ar-H), 5.08(1H, d, *J* =8.29 Hz, CH-OH), 4.54(1H, d, *J* =8.28 Hz, CH-Br), 4.25(2H, q, *J*=7.11 Hz, CH_2), 1.25(3H, t, *J*=7.12 Hz CH_3).

Example (iii)

Synthesis of (±)-methyl 3-hydroxy-2-iodo-3-phenylpropanoate of formula 2 where x=I and R'= CH_3.

To a stirred suspension of methyl cinnamate(3g, 20 mmol), $HIO_4.2H_2O$ (5.2g, 24 mmol) 12 ml water and 40 ml of acetonitrile, 1M sodium bisulphite solution (5.2g in 50ml) was added to the above mixture over a period of three-four hours with stirring and at 30°C. The reaction mixture was further stirred for 36 hrs till the reaction was complete (TLC monitored). The resulting solution was extracted with ethyl acetate (3x50ml), and combined organic layer was washed with aqueous sodium sulphite followed by drying over anhydrous sodium sulphate. The contents concentrated in vacuo to give a crude material, which was purified by crystallization (benzene: hexane, 1:1) to furnish compound of formula 2 m.pt 63°C, yield 3g (65%).

^{1}HNMR($CDCl_3$) δ: 7.35(5H, s, Ar-H), 5.05(1H, d, *J* =8.42 Hz, CH-OH), 4.55(1H, d, *J* =8.43 Hz, CH-Br), 3.75(3H, s, CH_3).

Example (iv)

Synthesis of (±)-ethyl 3-hydroxy-2-iodo-3-phenylpropanoate of formula 2 where x=I and R'= C_2H_5.

It was prepared from ethyl cinnamate.(3.26g, 20 mmol), $HIO_4.2H_2O$ (5.2g, 24 mmol) following the procedure given in example step 'a' (iii), yield 4g (77%), m.pt 79°C.

^{1}HNMR($CDCl_3$) δ: 7.38(5H, s, Ar-H), 5.05(1H, d, *J*=8.29 Hz, CH-OH), 4.55(1H, d, *J*=8.29 Hz, CH-Br), 4.25(2H, q, *J*=7.11 Hz, CH_2), 1.25(3H, t, *J*=7.12 Hz, CH_2CH_3).

Step 'b'

Example (i)

Synthesis of (±)-methyl 3-acetoxy-2-bromo-3-phenylpropanoate of formula 3 where x=Br and R'= CH_3.

A solution of **2** (2.59g, 10mmol) and acetic anhydride (12mmol) and dimethyl N,N-dimethyl aminopyridine (DMAP) (in catalytic amount) in 10 ml of dry dichloromethane was kept overnight at room temp. The reaction mixture was poured into ice-cold water and extracted with dichloromethane (3x100ml). The organic layer was washed, dried, and evaporated to furnish compound of formula **3**, which was purified by column chromatography (silica gel, ethyl acetate:hexane;3:97), in 90-95% yield, m.pt. 56°C

^{1}HNMR ($CDCl_3$)δ: 7.45(5H, s, Ar-H), 6.23(1H, d, *J*=10.00 Hz, CH-OAc), 4.58(1H, d, *J*=10.00 Hz, CH-Br), 3.83(3H, s, $COOCH_3$), 2.03(3H, s, $OCOCH_3$).

Example (ii)

Synthesis of (±)-ethyl 3-acetoxy-2-bromo-3-phenylpropanoate of formula 3 where x=Br and R'= C_2H_5.

It was prepared from **2** (2.73g, 10mmol) and acetic anhydride (12mmol) and N,N-dimethyl aminopyridine (DMAP), in catalytic amount) following the procedure given in step 'b' example (i) in 90-95% yield.

^{1}HNMR ($CDCl_3$)δ: 7.50(5H, s, Ar-H), 6.23(1H, d, *J*=10.50 Hz, CH-OAc), 4.53(1H, d, *J*=10.50 Hz, CH-Br), 4.33(2H, q, *J*=7.11 Hz CH_2), 2.06(3H, s, $OCOCH_3$), 1.33(3H, t, *J*=7.12 Hz CH_2CH_3).

Example (iii)

Synthesis of (±)-methyl 3-acetoxy-2-iodo-3-phenylpropanoate of formula 3 where x=I and R'= CH_3.

It was prepared from 2 (3.06g, 10mmol) and acetic anhydride (12mmol) and N,N-dimethyl aminopyridine (DMAP), in catalytic amount) following the procedure given in step 'b' example (i) in 90-95% yield, m.pt, 58°C.

^{1}HNMR($CDCl_3$) δ: 7.40(5H, s, Ar-H), 6.15(1H, d, *J*=10.75 Hz, CH-OAc), 4.62(1H, d, *J*=10.75 Hz, CH-Br), 3.17(3H, s, $COOCH_2$), 2.00(3H, s, CH_3).

Example (iv)

Synthesis of (±)-ethyl 3-acetoxy-2-iodo-3-phenylpropanoate of formula 3 where x=I and R'= C_2H_5.

It was prepared from 2 (3.19g, 10mmol) and acetic anhydride (12mmol) and N,N-dimethyl aminopyridine (DMAP), in catalytic amount) following the procedure given in step 'b' example (i) in 90-95% yield.

^{1}HNMR($CDCl_3$) δ: 7.50(5H, s, Ar-H), 6.15(1H, d, *J*=10.50 Hz, CH-OAc), 4.62(1H, d, *J*=10.50 Hz, CH-Br), 4.25(2H, q, *J*=7.11 Hz $COOCH_2$), 2.06(3H, s, $OCOCH_3$), 1.25(3H, t, *J*=7.12 Hz CH_2CH_3).

Step 'c'

Example (i)

Preparation of (-)-methyl (2*R*,3*R*)-2-bromo-3-hydroxy-3-phenylpropanoate of formula 4 by kinetic resolution, where x=Br and R'= CH_3.

(±)-Methyl 3-acetoxy-2-bromo-3-phenylpropanoate (800mg) of formula 3 where x=Br and R'= CH_3, was added to biphasic system of aqueous phosphate buffer (16ml, 0.1M. pH 7.0) and toluene (1.6ml). To the above solution was added crude dry powder of lipase *Aspergillus niger* (Amano AS, 400mg, 12-15 units/mg) with the continuous stirring and maintaining pH 7.0 by addition of 0.5N sodium hydroxide solution. During the course of the reaction temperature was maintained at 30°C. The progress of the reaction was monitored after every six hours by TLC and HPLC. After completion of the reaction (48 hrs., approx., 43% conversion), the reaction was terminated by centrifuging the mixture at 10,000 to 15,000g to remove enzyme and the suspended particles. The clear solution and the centrifuged mass was extracted separately with ethyl acetate (3 x 20ml). The organic layer was combined and washed with water. The combined solvent layer

was then dried and evaporated under reduced pressure to furnish a mixture comprising hydrolysed alcohol and unhydrolysed ester which were separated by column chromatography over silica gel using hexane:ethyl acetate (97:3) as eluent to furnish (-)-methyl (2*R*,3*R*)-2-bromo-3-hydroxy-3-phenylpropanoate of formula 4 (250mg, 85%) having enantiomeric purity (EE)92%, $[\alpha]_D^{23}$ -19.7^0 (c,1,$CHCl_3$) and unhydrolysed ester (+)-methyl (2*S*,3*S*)-3-acetoxy-2-bromo-3-phenylpropanoate of formula **5** (378mg, 83%) having enantiomeric purity (EE)70% (chiral HPLC), $[\alpha]_D^{25}$ $+36.4^0$ (c,1,$CHCl_3$).

Example (ii)

Preparation of (-)-ethyl (2*R*,3*R*)-2-bromo-3-hydroxy-3-phenylpropanoate of formula 4 by kinetic resolution, where x=Br and R'= C_2H_5.

It was prepared from (±)-ethyl 3-acetoxy-2-bromo-3-phenylpropanoate (800mg) of formula **3**, phosphate buffer (16ml, 0.1M. pH 7.0), toluene (1.6ml) and crude dry powder of lipase *Aspergillus niger* (Amano AS, 400mg, 12-15 units/mg) following the procedure given in step 'c' example (i). After 48 hrs. (45% conversion) the hydrolyzed alcohol was obtained (256mg, 82%), having enantiomeric purity (EE) 86%, $[\alpha]_D^{25}$ -14.8^0 (c,1,$CHCl_3$) and unhydrolysed ester (+)-ethyl (2*S*,3*S*)-3-acetoxy-2-bromo-3-phenylpropanoate of formula **5** (375mg, 85%) having enantiomeric purity (EE)73%(chiral HPLC), $[\alpha]_D^{25}$ $+37.5^0$ (c,1,$CHCl_3$).

Example (iii)

Preparation of (-)-methyl (2*R*,3*R*)-3-hydroxy-2-iodo-3-phenylpropanoate of formula 4 by kinetic resolution, where x=I and R'= CH_3.

It was prepared from (±)-Methyl 3-acetoxy-2-iodo-3-phenylpropanoate (800mg) of formula **3**, phosphate buffer (16ml, 0.1M. pH 7.0), toluene (1.6ml) and crude dry powder of lipase *Aspergillus niger* (Amano AS, 400mg, 12-15 units/mg) following the procedure given in step 'c' example (i). After 36 hrs. (44% conversion) the hydrolyzed alcohol was obtained (274mg, 88%), having enantiomeric purity (EE) 94%, $[\alpha]_D^{25}$ 0.0^0 (c,1,$CHCl_3$); -3.0^0 (c 1, MeOH) and

unhydrolysed ester (+)-methyl-(2*S*,3*S*)-3-acetoxy-2-iodo-3-phenylpropanoate of formula 5 (415mg, 92%) having enantiomeric purity (EE)76%(chiral HPLC), $[\alpha]_D^{25}$ $+48.0^0$ (c,1,$CHCl_3$).

Example (iv)

Preparation of (-)-ethyl (2*R*,3*R*)-3-hydroxy-2-iodo-3-phenylpropanoate of formula 4 by kinetic resolution, where x=I and R'= C_2H_5.

It was prepared from (±)-Ethyl 3-acetoxy-2-iodo-3-phenylpropanoate (800mg) of formula 3, phosphate buffer (16ml, 0.1M. pH 7.0), toluene (1.6ml) and crude dry powder of lipase *Aspergillus niger* (Amano AS, 400mg, 12-15 units/mg) following the procedure given in step 'c' example (i). After 40 hrs. (40% conversion) the hydrolyzed alcohol was obtained (250mg, 88%), having enantiomeric purity(EE)95%, $[\alpha]_D^{25}$ -9.3^0 (c,1,$CHCl_3$) and unhydrolysed ester (+)-ethyl(2*S*,3*S*)-3-acetoxy-2-iodo-3-phenylpropanoate of formula 5 (440mg, 91%) having enantiomeric purity (EE)60%(chiral HPLC), $[\alpha]_D^{25}$ $+36.0^0$ (c,1,$CHCl_3$) .

Example (v)

Preparation of (+)-methyl (2*S*,3*S*)-3-acetoxy-2-bromo-3-phenylpropanoate of formula 5 by double kinetic resolution, where x=Br and R'= CH_3.

It was prepared from optically enriched (+)-Methyl (2*S*,3*S*)-3-acetoxy-2-bromo-3-phenylpropanoate (EE, 70%, 350mg) of formula 5, phosphate buffer (7ml, 0.1M. pH 7.0), toluene (0.7ml) and crude dry powder of lipase *Aspergillus niger* (Amano AS, 100mg, 12-15 units/mg) following the procedure given in step 'c' example (i). After completion of the reaction (72 hrs.), the unhydrolyzed ester on separation (290mg) was found to have enantiomeric excess (EE)>99%(chiral HPLC), $[\alpha]_D^{25}$ $+52.0^0$ (c,1, $CHCl_3$)

Example (vi)

Preparation of (+)-ethyl (2*S*,3*S*)-3-acetoxy-2-bromo-3-phenylpropanoate of formula 5 by double kinetic resolution, where x=Br and R'= C_2H_5.

It was prepared from optically enriched ethyl (2S,3S)-3-acetoxy-2-bromo-3-phenylpropanoate (EE 73%, 350mg) of formula **3**, phosphate buffer (7ml, 0.1M. pH 7.0), toluene (0.7ml) and crude dry powder of lipase *Aspergillus niger* (Amano AS, 100mg, 12-15 units/mg) following the procedure given in step 'c' example (i). After 65 hrs the unhydrolyzed ester on separation (290mg) was found to have enantiomeric excess (EE)98%(chiral HPLC), $[\alpha]_D^{25}$ +50.5^0 (c,1, $CHCl_3$).

Example (vii)

Preparation of (+)-methyl (2S,3S)-3-acetoxy-2-iodo-3-phenylpropanoate of formula 5 by double kinetic resolution, where x=I and R'= CH_3.

It was prepared from optically enriched methyl (2S,3S)-3-acetoxy-2-iodo-3-phenylpropanoate (EE 76%, 350mg) of formula **3**, phosphate buffer (7ml, 0.1M. pH 7.0), toluene (0.7ml) and crude dry powder of lipase *Aspergillus niger* (Amano AS, 100mg, 12-15 units/mg) following the procedure given in step 'c' example (i). The unhydrolyzed ester of formula **5** (300mg) was found to have enantiomeric excess (EE)94%(chiral HPLC), $[\alpha]_D^{25}$ +59.0^0 (c,1, $CHCl_3$).

Example (viii)

Preparation of (+)-ethyl (2S,3S)-3-acetoxy-2-iodo-3-phenylpropanoate of formula 5 by double kinetic resolution, where x=I and R'= C_2H_5.

It was prepared from optically enriched methyl (2S,3S)-3-acetoxy-2-iodo-3-phenylpropanoate (EE 60%, 350mg) of formula **3**, phosphate buffer (7ml, 0.1M. pH 7.0), toluene (0.7ml) and crude dry powder of lipase *Aspergillus niger* Amano AS (100mg, 12-15 units/mg) The unhydrolyzed ester (315mg)(72hrs.) was found to have enantiomeric excess (EE)73%(chiral HPLC), $[\alpha]_D^{25}$ +43.5^0 (c,1, $CHCl_3$).

Example (ix)

Preparation of (-)-methyl (2*R*,3*R*)-2-iodo-3-hydroxy-3-phenylpropanoate of formula 4 by kinetic resolution in presence of a cosolvent acetonitrile, where x=I and R'= CH_3.

(±)-Methyl 3-acetoxy-2-iodo-3-phenylpropanoate (200mg) of formula 3 was added to biphasic system of aqueous phosphate buffer (3.6ml, 0.1M. pH 7.0) and acetonitrile (0.4ml). To the above solution crude dry powder of lipase *Aspergillus niger* (Amano AS, 100mg, 12-15 units/mg) was added with the continuous stirring and maintaining pH 7.0 by addition of 0.5N sodium hydroxide solution. During the course of the reaction temperature was maintained at 30°C. The progress of the reaction was monitored after every six hours. After the completion of the reaction (9 hrs., approx., 43% conversion), the reaction was terminated by centrifuging the mixture at 10,000 to 15,000g to remove enzyme and the suspended particles. The clear solution and the centrifuged mass was extracted separately with ethyl acetate (3X30 ml). The organic layer was combined and washed with water. The combined solvent layer was then dried and evaporated under reduced pressure to furnish a mixture comprising hydrolysed alcohol and unhydrolysed ester which were separated by column chromatography over silica gel using hexane: ethyl acetate (97:3) as eluent to furnish methyl (2*R*,3*R*)-3-hydroxy-2-iodo-3-phenylpropanoate of formula 4 (65mg, 85%) having enantiomeric purity (EE)92% and unhydrolysed ester methyl (2*S*,3*S*)-3-acetoxy-2-iodo-3-phenylpropanoate of formula 5 (92 mg, 77%), enantiopurity (EE) 62% determined by chiral HPLC.

Step 'e'

Example (i)

Preparation of (+)-methyl (2S,3R)-phenylglycidate of the formula 7 where R'= CH_3

0.2 ml of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) was added to a solution of optically enriched halohydrin (200 mg) of formula 4 in methanol (4ml) at 20° C for 5 minutes. The solvent was removed under reduced pressure and the reaction mixture was diluted with 10 ml water and extracted with ethyl acetate and solvent removed to furnish compound of formula 7; recovery 98mg, $[\alpha]_D^{25}$ +155.0° (c,1, $CHCl_3$)

Step 'f'

Example (i)

Preparation of (+)-methyl (2S,3S)-2-halo-3-hydroxy-3-phenylpropanoate of formula 6 where R'= CH_3

Alkyl (2S,3S)-3-acetoxy-2-holo-3-phenylpropanoate of formula 5 (200mg) was dissolved in 4ml of methanol and 0.2ml of 2N HCl was added and the reaction mixture was stirred at room temperature for 24 hrs till the complete conversion of the compound of formula **5** to the compound of the formula **6**. Excess of solvent was evaporated and the reaction mixture was diluted with 10 ml water and extracted with ethyl acetate, and solvent removed to furnish compound of formula 6, recovery 150mg.

Example (ii)

Preparation of (-)-methyl (2R,3S)-phenylglycidates of the formula 8 where R'= CH_3

It was prepared from **6** following the procedure given in step 'f' example (i) for compound 7. $[\alpha]_D^{25}$ -153.0^0 (c,1, $CHCl_3$)

We claim

1. A novel process for the chemoenzymatic synthesis of two enantiomers of trans alkyl phenylglycidate i.e. alkyl(2*S*,3*R*)-phenylglycidate and alkyl(2*R*,3*S*)-phenylglycidate of formulae 7 and 8 respectively,wherein the said process of their synthesis comprises cohalogenation reaction of alkyl cinnamate of formula 1 where R' represents C-1 to C-5 alkyl group to form trans 2-halo-3-hydroxy-3-phenylpropanoates of formula 2 where x represents Br or I, then converting the halohydrins of formula 2 to corresponding alkyl acylates of formula 3 where x and R' represents as above, subsequently incubating the acyl derivatives of alkyl 2-halo-3-hydroxy-3-phenylpropanoates of formula 3 with crude dry powder of lipase from *Aspergillus niger* in an aqueous buffer phase in presence of an organic solvent, thereafter separating the hydrolysed halohydrins i.e. alkyl(2R,3R) 2-halo-3-hydroxy-3-phenylpropanoates of formula 4 and unhydrolysed ester i.e. alkyl (2S,3S) 2-halo-3-acyloxy-3-phenylpropanoates of formula 5 from the mixture by conventional method, if required then again incubating the optically enriched acyl derivatives of formula 5 with crude dry powder of lipase from *Aspergillus niger* in an aqueous buffer phase in presence of an organic solvent to further improve the enantiopurity, followed by reaction of optically enriched products of formula 5 with an acid to furnish optically enriched alkyl (2S,3S) 2-halo-3-hydroxy-3-phenylpropanoate of formula 6 and finally treating the compounds of formulae 4 and 6 with an alkali in an organic or aqueous phase to furnish optically enriched alkyl(2*S*,3*R*)-phenylglycidate and alkyl(2*R*,3*S*)-phenylglycidate of formulae 7 and 8 respectively

2. A novel process as claimed in claim 1 wherein the halogenating agent used for the preparation of trans halohydrin of formula 2 are selected from N-halosuccinimide such as N-bromosuccinimide, N-iodosuccinimide or sodium bromate, periodic acid, 1,3-dibromo-5,5-dimethyl hydantoin, iodine, bromine and the like.

3. A process as claimed in claims 1and 2 wherein the halohydroxylation process is effected in aqueous or in an organic phase or aqueous organic phase where

organic phase may be selected from water miscible solvents such as acetone, tetrahydrofuran, dioxane, dimethyl formamide, methanol and the like .

4. A process as claimed in claim 1 to 3 wherein the halohydroxylation process is effected at a temperature between 0-60^{0}C.

5. A process as claimed in claim 2 wherein the acylating agent is selected from acyl anhydrides such as acetic anhydride; propionic anhydride, butyric anhydride or corresponding acyl chlorides in presence of bases like pyridine, N,N-dimethyl aminopyridine (DMAP) and the like

6. A process as claimed in claim 1 wherein the kinetic resolution process is effected by crude dry powder of lipase from *Aspergillus niger.*

7. A process as claimed in claims 1 and 5 wherein for the stereospecific hydrolysis of alkyl acylates of formula 3, the aqueous phosphate buffer is selected from buffers such as phosphate, tris and acetic acid and the like at pH in the range of 5 to 7.5 and temperature 10-40^{0}C.

8. A process as claimed in claims 5 and 6 wherein stereospecific hydrolysis is most suitably carried out in presence of an organic cosolvent such as hexane, toluene, dichloromethane, acetone, acetonitrile, dimethylformamide, dimethyl sulphoxide, methanol, ethanol and the like at 10-90% concentration.

9. A process as claimed in claims 5 to 7 wherein the stereospecific hydrolysis is effected suitably at a temperature 30^{0}C.

10. A process as claimed in claims 1 wherein the cyclisation to optically enriched glycidate of formula 7 is effected most suitably in presence of an organic or inorganic base such as sodium hydroxide, sodium carbonate and the like and organic bases are selected from triethylamine, piperidine,1,4-diazabicyclo[2,2,2]octane (DABCO), 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) and the like.

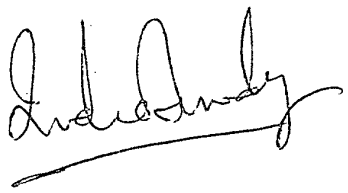
11. A process as claimed in claims 1 and 9 wherein the cyclisation to optically enriched glycidate of formula 8 is effected most suitably in presence of an organic or inorganic acid such as hydrochloric acid , sulfuric acid or trifluoro acetic acid, boron trifluoride (BF_3) and the like.

12. A process as claimed in claims 1 to 10 wherein the products of the formula 7 has enantiomeric excess between 86-95%.

13. A process as claimed in claims 1 to 11 wherein the products of the formula 8 has enantiomeric excess between 60-99.5%.

14. A stereospecific process for the preparation of alkyl(2*S*,3*R*)-phenylglycidate and alkyl(2*R*,3*S*)-phenylglycidate of formulae 7 and 8 respectively shown in the drawing accompanying the specification substantially as herein described with reference to examples.

Dated the 20th day of March 2003



Dr. (Smt.
वैज्ञानिक
आई.पी.सेल.डी.
I. P.

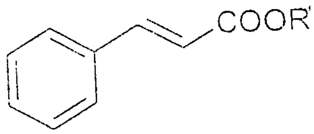
THIS PAGE BLANK (USPTO)

COUNCIL OF SCINTIFIC &INDUSTRIAL RESEARCH

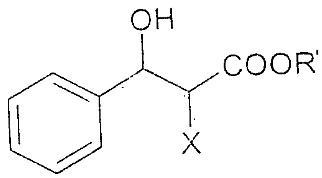
Sheet No.1

0275-DEL-03

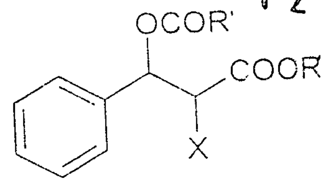
12 MAR 2003



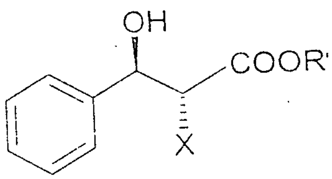
(1)



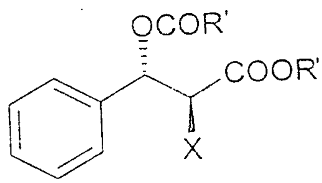
(2)



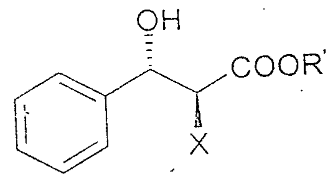
(3)



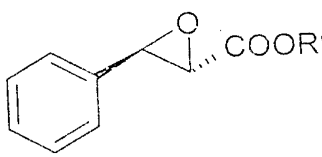
(4)



(5)

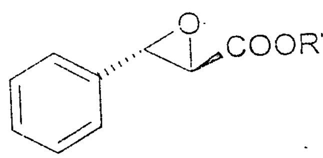


(6)



(7)

(2S,3R)



(8)

(2R,3S)

ORIGINAL

R' = CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}

X = Br, I

Dr. (Smt.) I... Dwivedy

वैज्ञानिक/Scientist

New Delhi

THIS PAGE BLANK (USPTO)

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☒ OTHER: Not clear

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

THIS PAGE BLANK (USPTO)